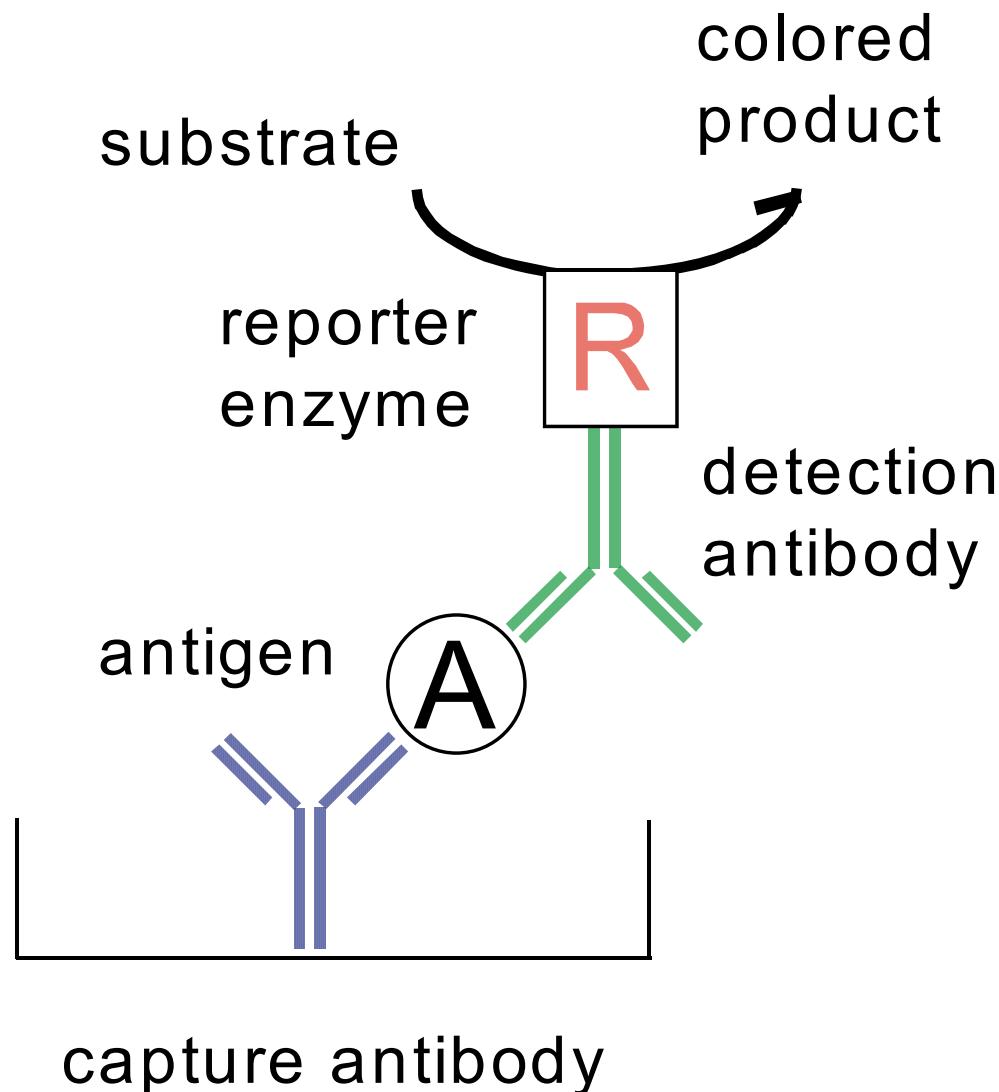


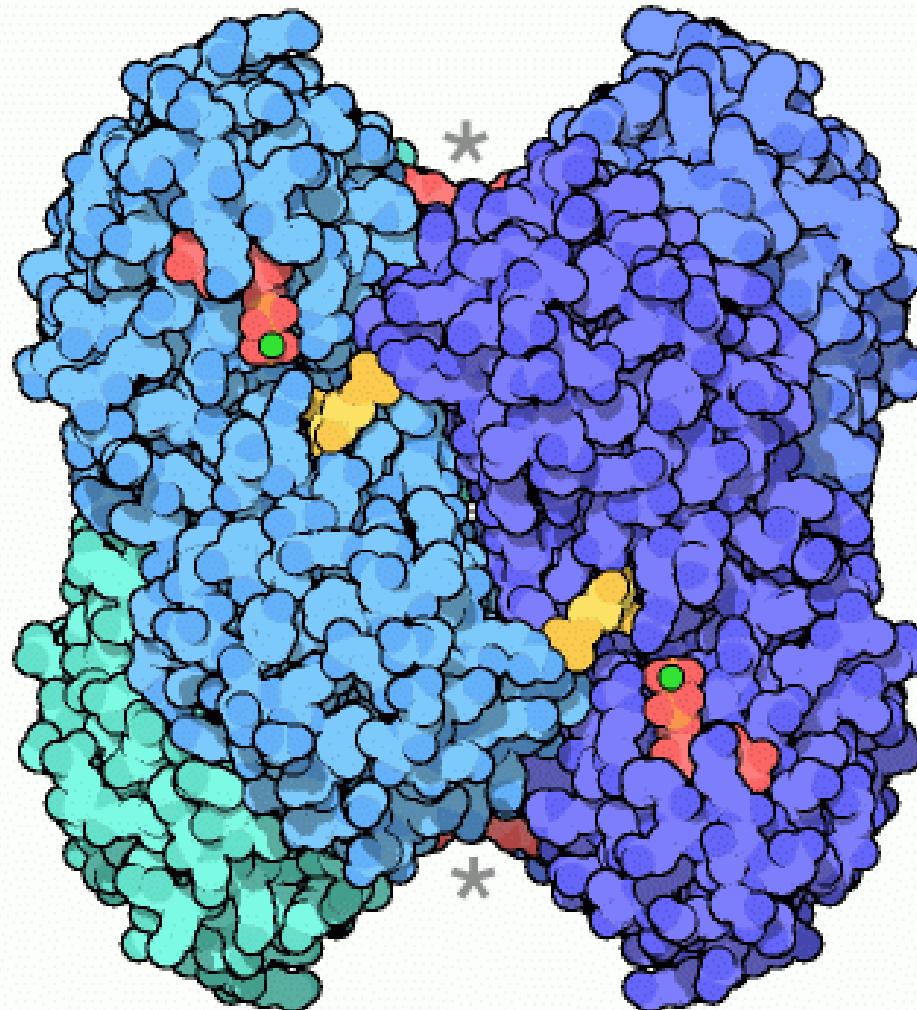
Engineered reporter proteins as biosensors

Ichiro Matsumura
Nordic Research Networking
Meeting, May 11, 2006

Enzyme-Linked ImmunoSorbent Assay



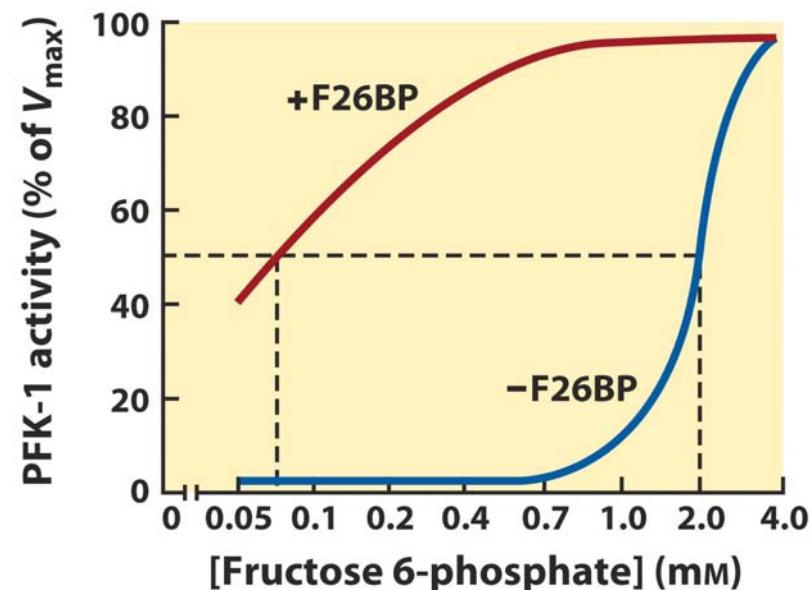
Nature is good at designing molecular switches



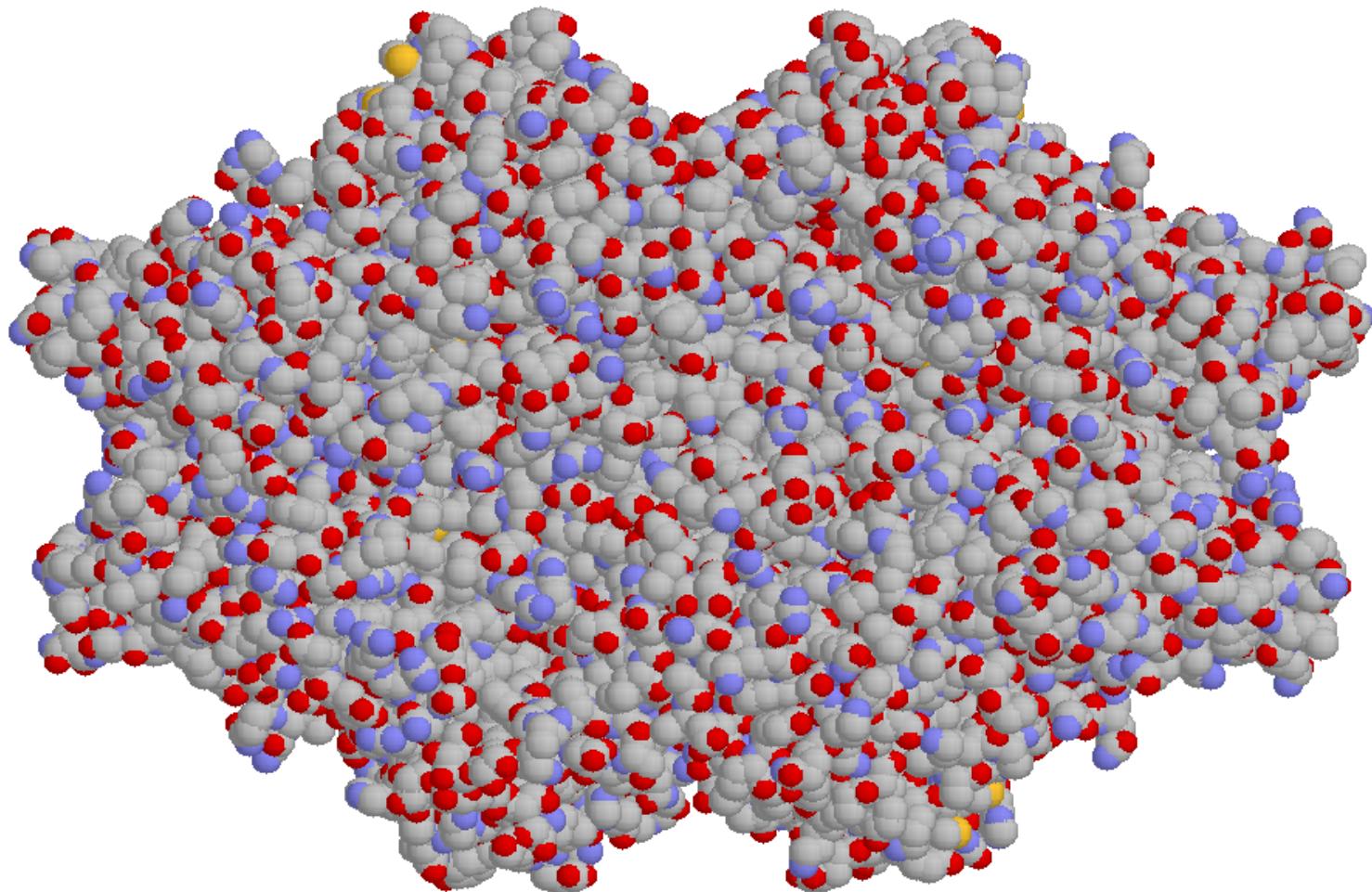
phosphofructokinase

substrate analogue

effectors

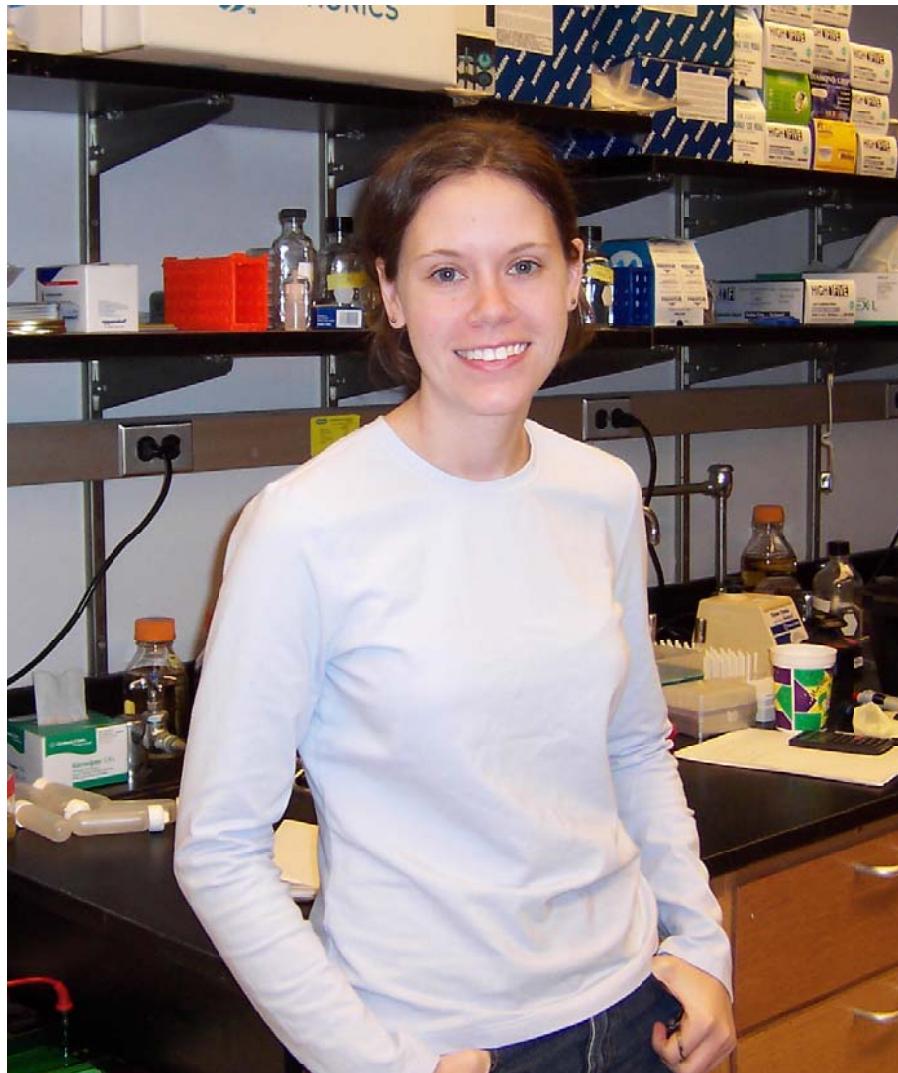


Human protein engineers are
generally not so good.



reporter enzyme beta-glucuronidase

Melissa Geddie re-designs GUS.

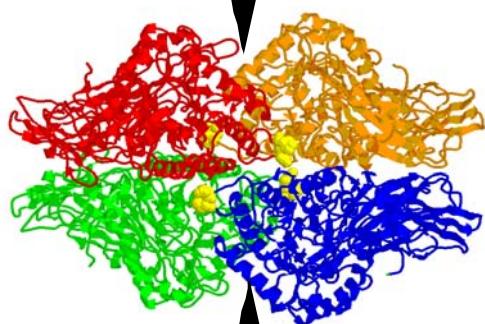


Her sensors exhibit:

- effector-dependence
- ~70-fold activation
- high sensitivity
- *ex vivo* function
- modularity

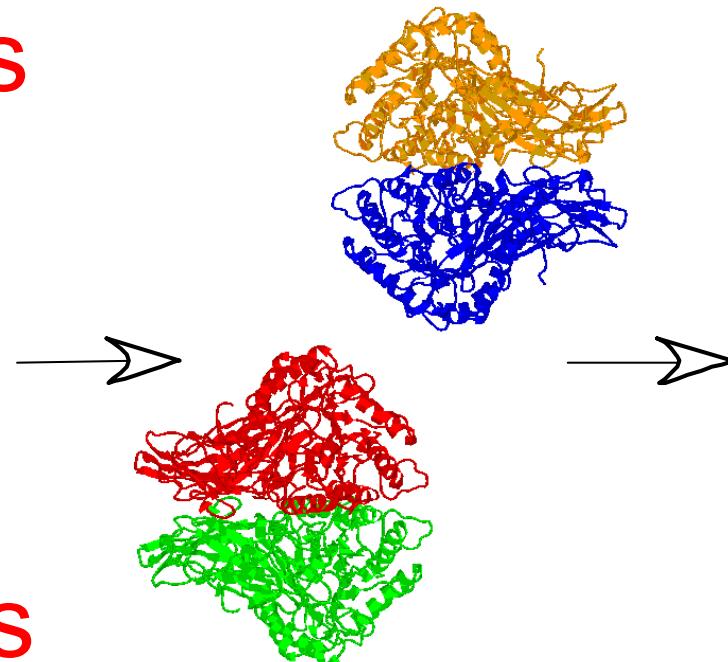
Melissa's simple but effective design strategy

mutagenesis

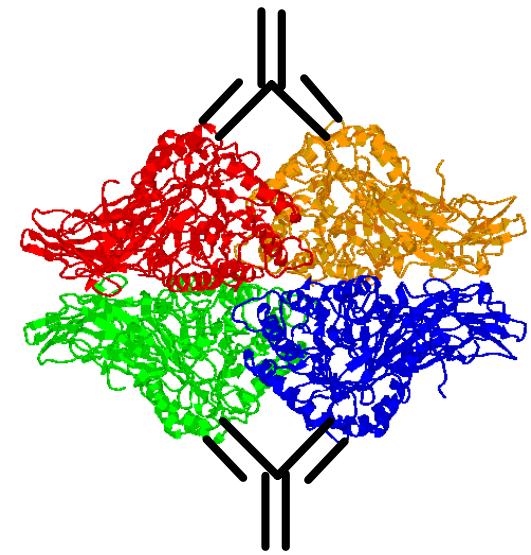


mutagenesis

wild-type
tetramer



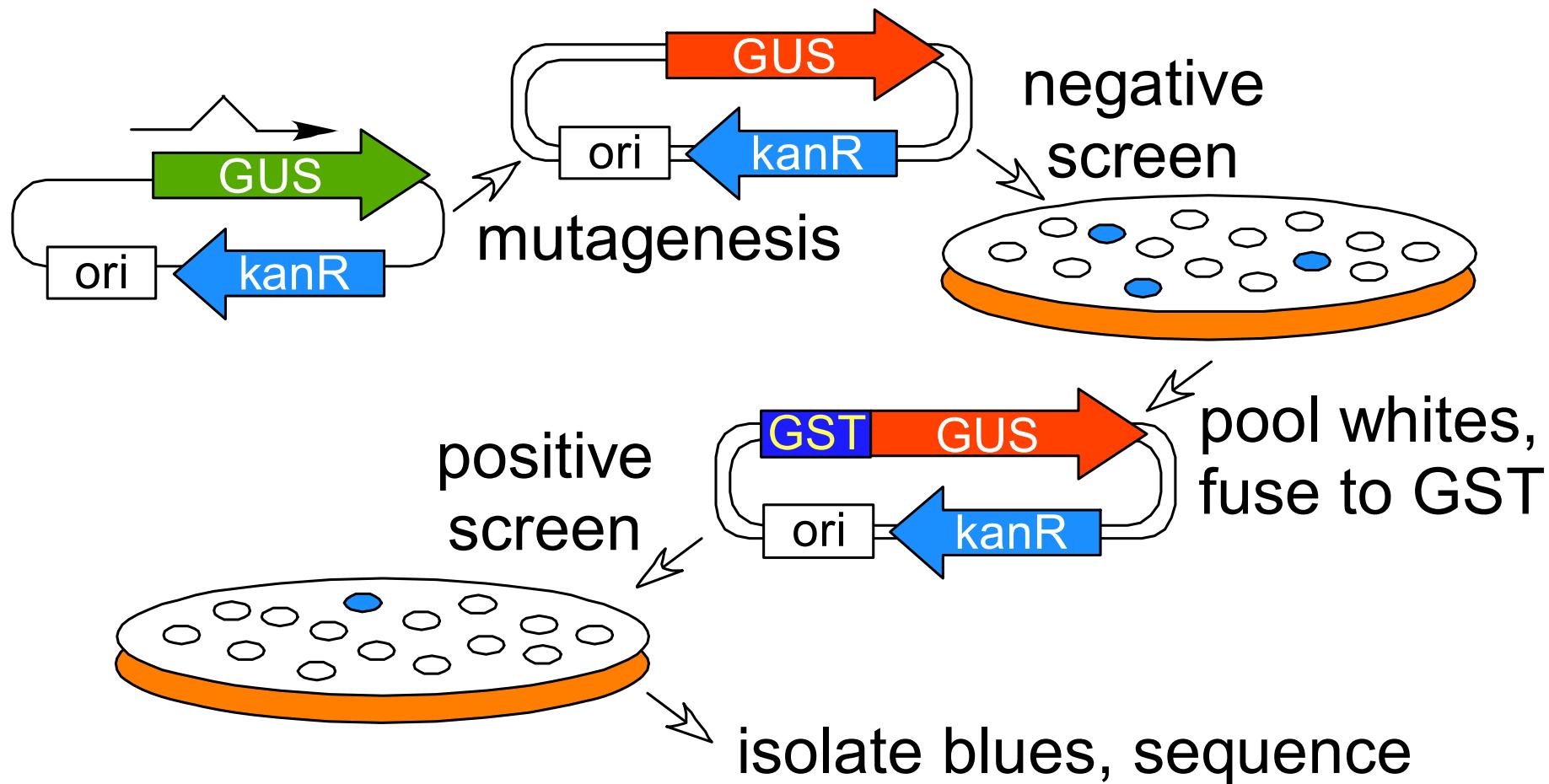
engineered
dimers



antibody

induced
tetramer

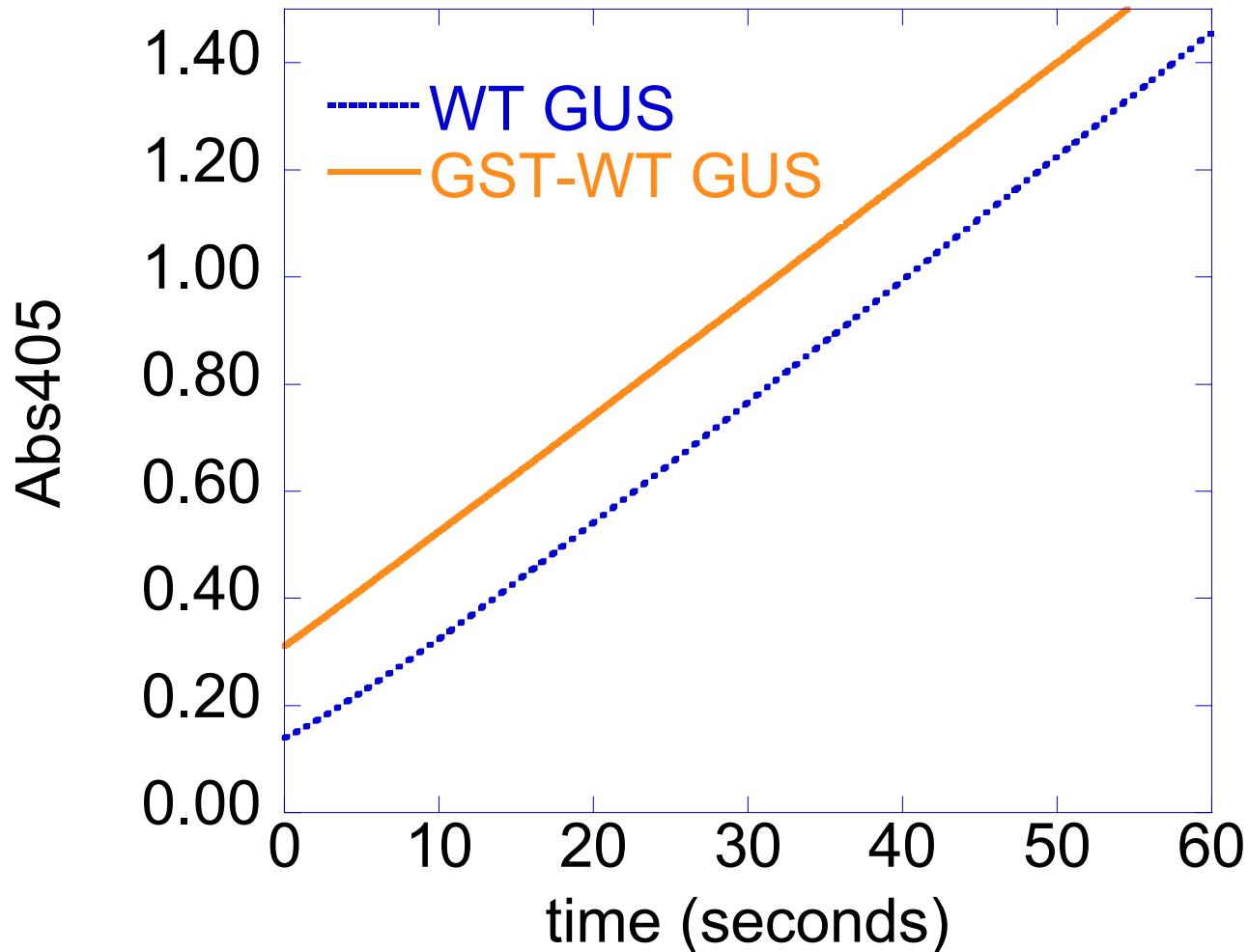
Two step-screen for GST-dependent GUS alleles



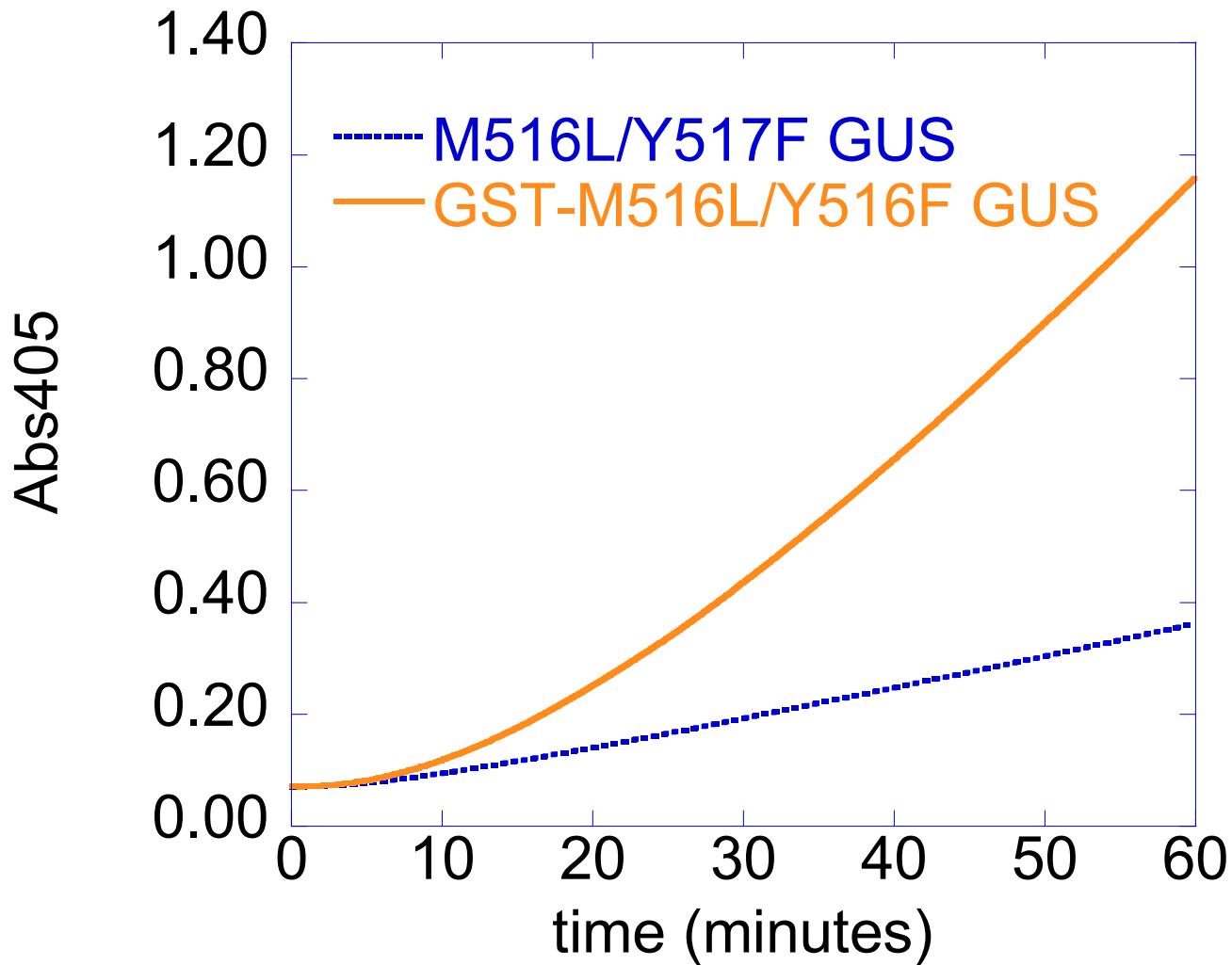
GST-dependent GUS variants

Wild-type	Met 516	Tyr 517
1-22	Cys	Val
1-26	Tyr	Phe
1-29	Phe	Tyr
2-10	Ile	Ile
2-17	Leu	Phe
2-24	Leu	Phe
3-7	Ile	Ile
3-18	Gln	Arg

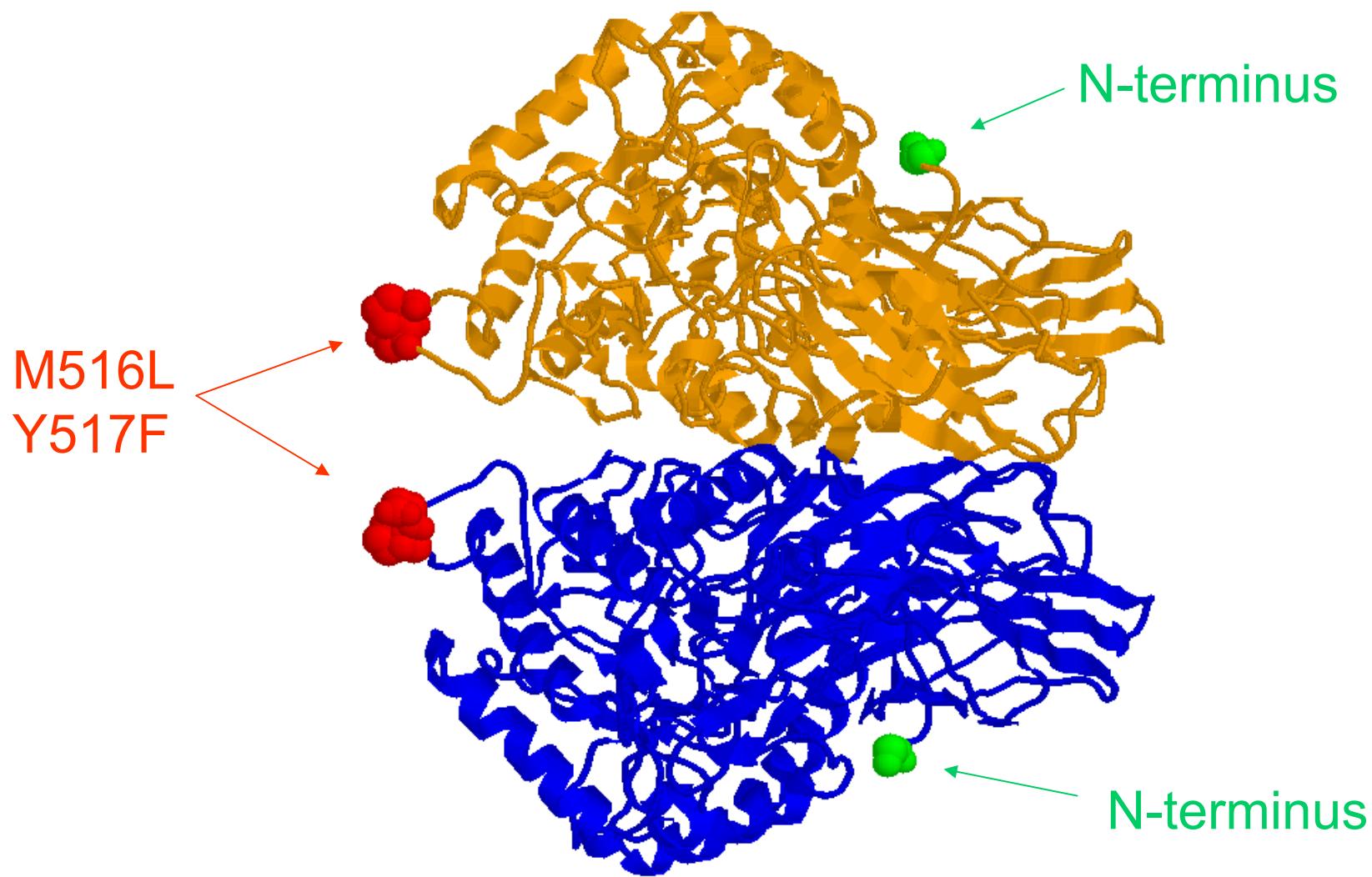
GST fusion does not affect wild-type GUS



GST fusion activates mutant GUS

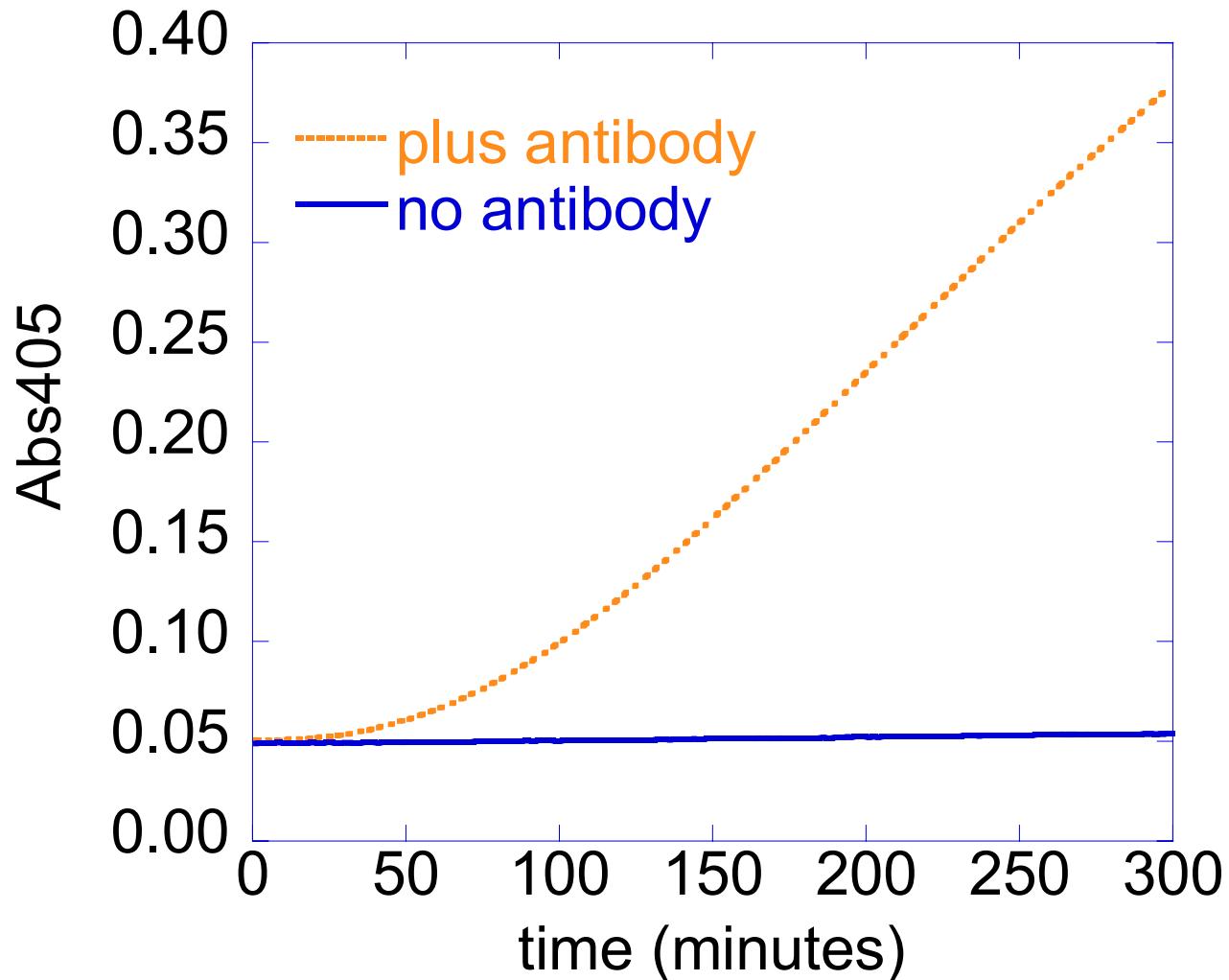


HA tagging of mutant GUS



Proof of principle

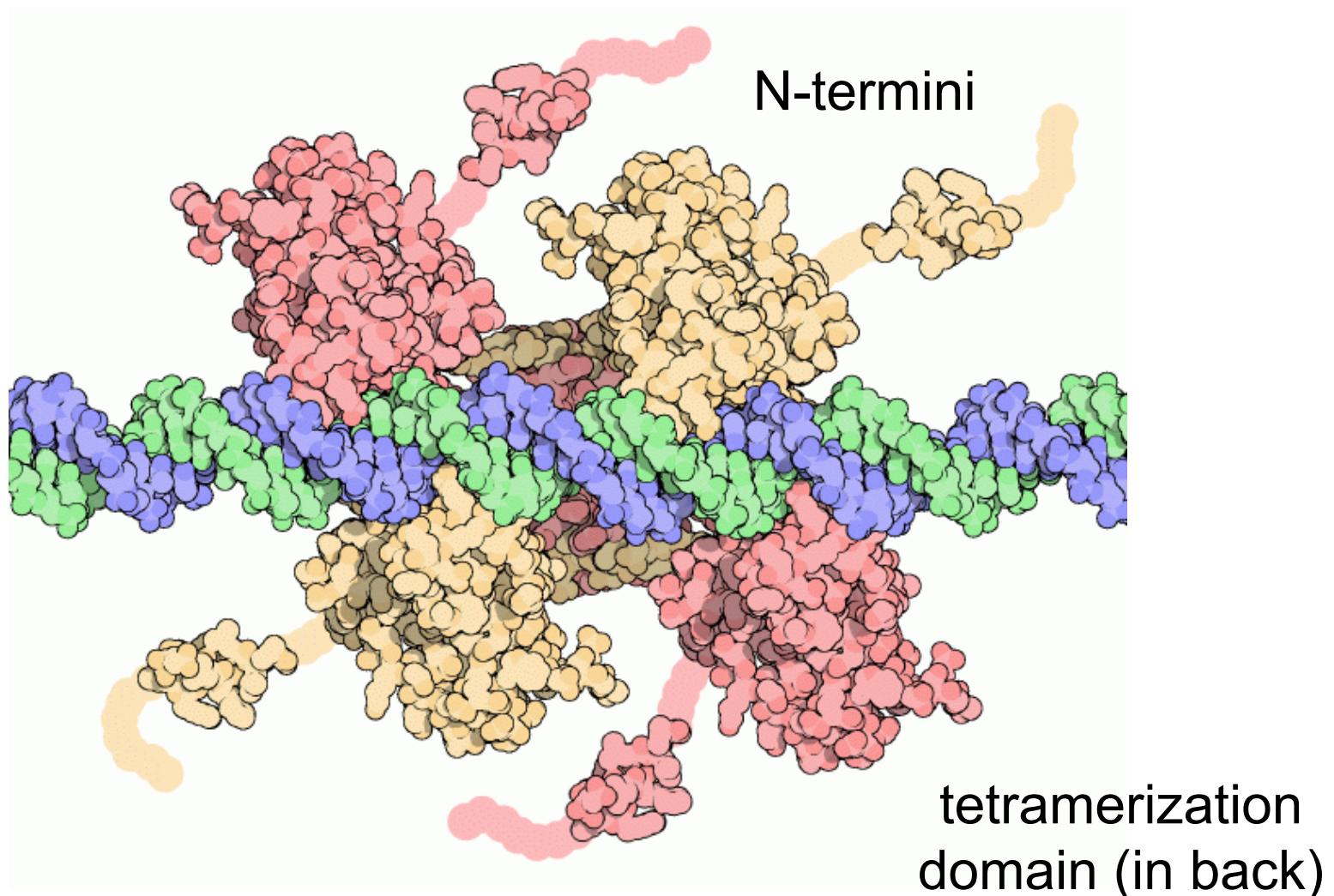
anti-HA antibody activates HA-M516L/Y517F-GUS



Generalizing the induced oligomerization strategy

- Detection of other targets
 - Other peptide epitopes (suggestions?)
 - Antibody-GUS fusions
- Other reporters
 - Transcription factor p53
 - Glucose dehydrogenase

Transcription factor p53 binds DNA as a tetramer

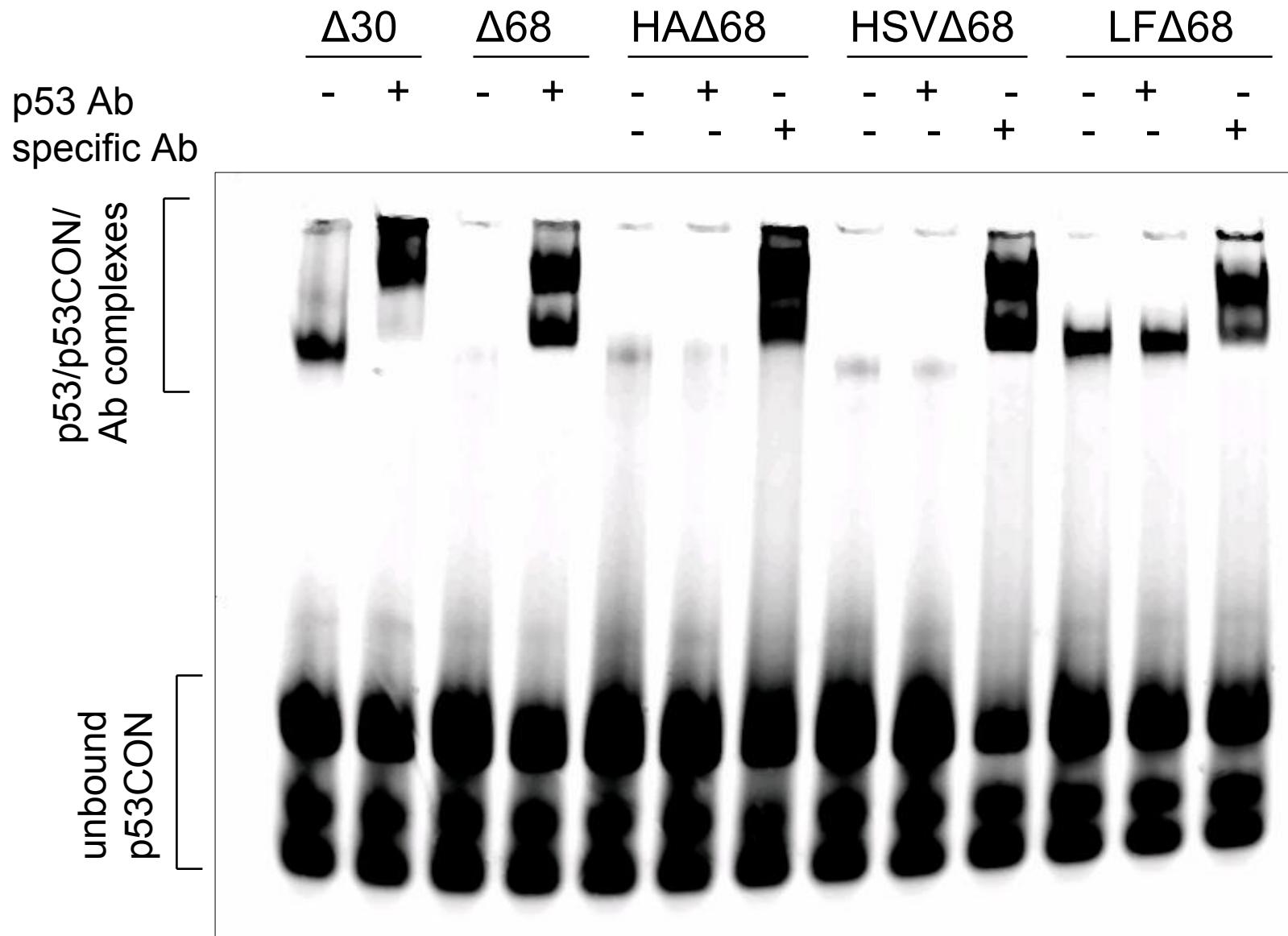


Peptide epitopes fused to p53

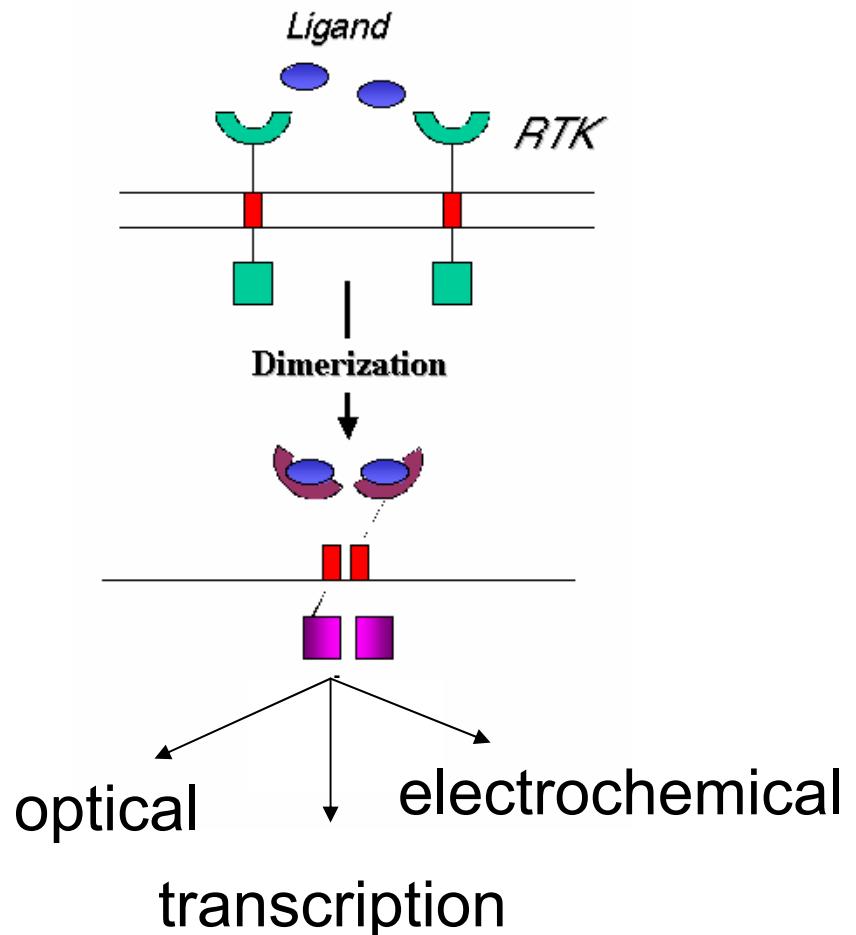
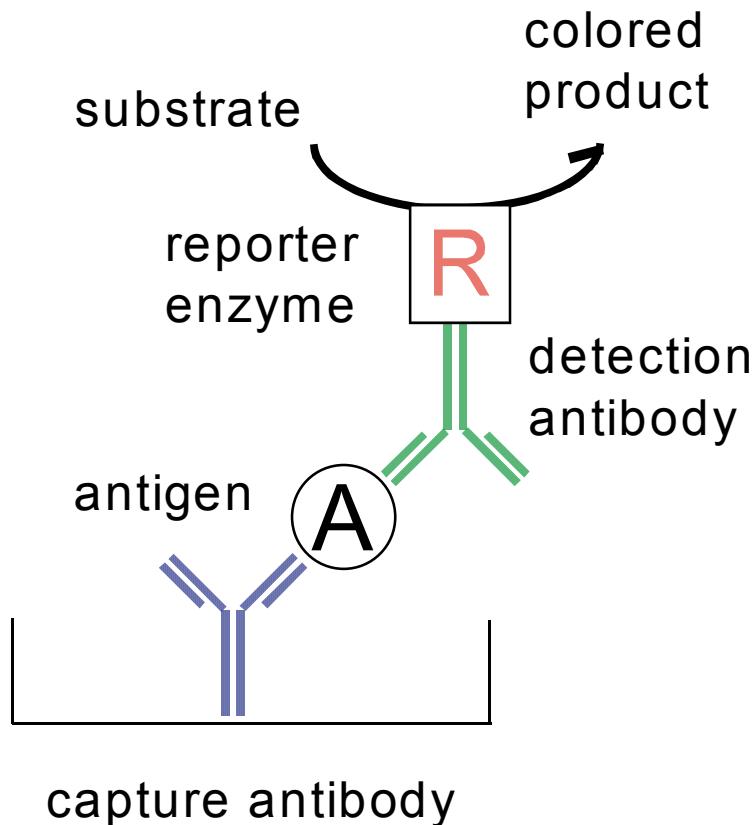
Antibody	Epitope	Origin
pAb1801	SPDDIENWFT	native p53
anti-HA	YPYDVPDYA	Influenza hemagglutinin
anti-HSV	QPELAPEDPED	herpes simplex virus
anti-LF	STDHAERLKVKQKNA	<i>B. anthracis</i> lethal factor

The C-terminal tetramerization domain was also deleted.

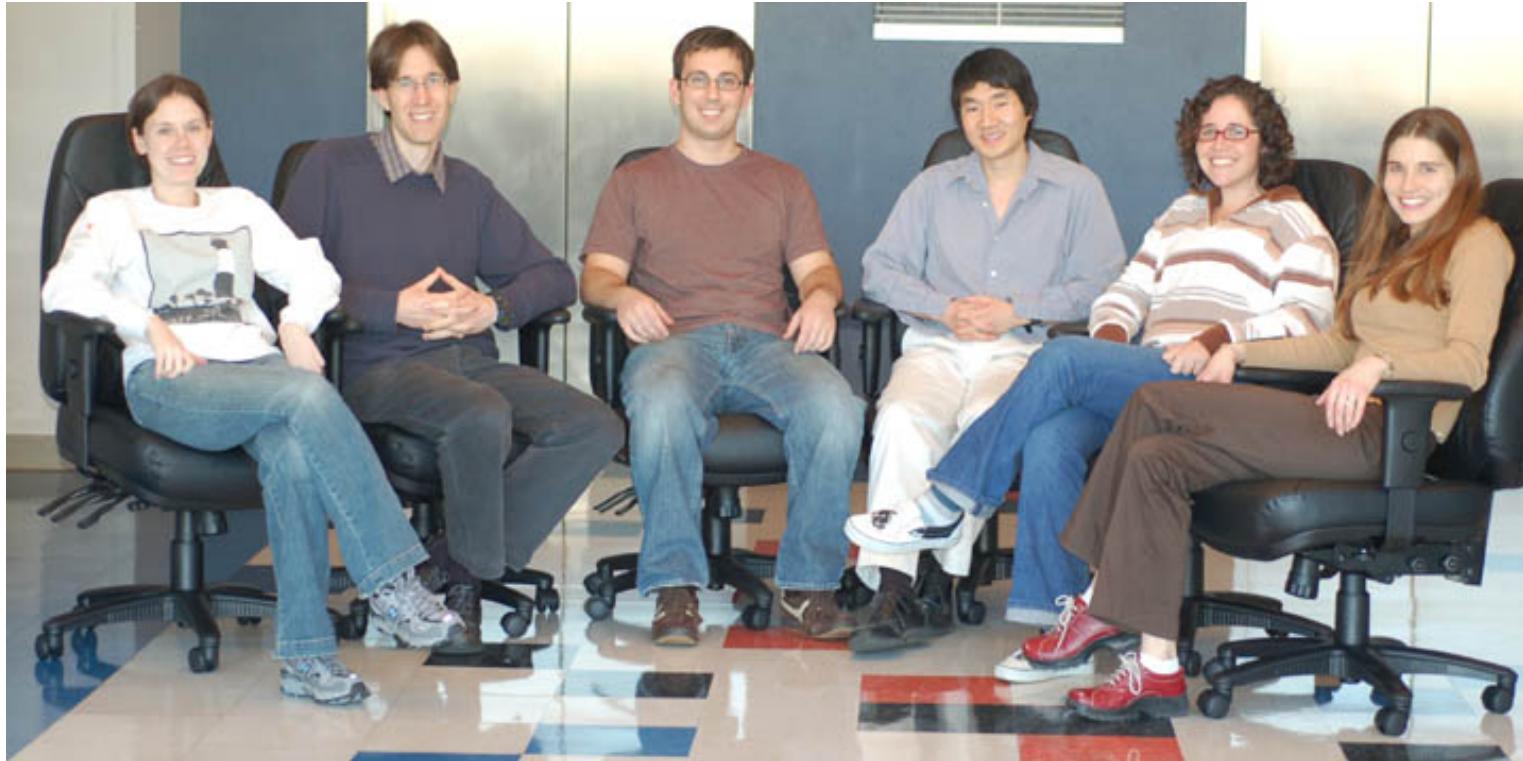
p53 activates with different Abs



"Smart" proteins could streamline diagnostic assays



Kiitos!



Melissa Geddie: molecular switches

Wayne Patrick: proteome evolution

Erik Quandt: protease evolution

Dina Greene: RuBisCO evolution

Taryn O'Loughlin: protease inhibitors

NIH, NSF (funding)

NIAIDS/NIH and Tekes